FINAL REPORT

South Carolina State Wildlife Grant SC-T-F13AF01208
South Carolina Department of Natural Resources
October 1, 2013 – September 30, 2016

Project Title: An Evaluation of Culturing Carolina Diamondback Terrapins for Responsible Stock Enhancement

<u>Objective 1 (Years 1 – 3):</u> Capture gravid female diamondback terrapins and collect eggs. Assess reproductive parameters of the diamondback terrapin population in the Charleston Harbor Estuary.

<u>Accomplishments</u>: Adult diamondback terrapins were captured in the Charleston Harbor Estuarine system during routine trammel net surveys, targeted terrapins trips, and on land while moving to nesting grounds. Terrapins were brought back to the laboratory to be sexed, measured (head width, carapace length, carapace width, plastron length, shell depth), weighed, and marked on three unique marginal scutes using an alphabetical code (A through X beginning on the right marginal scute and moving clockwise). All females were ultrasounded to detect the presence of follicles or eggs; those that had either follicles or shelled eggs were held in one of two outdoor tank systems (flow-through Charleston Harbor water) and all remaining females and males were released at the site of capture. The females retained for egg harvest were fed a natural diet (fish, snails, mussels and crabs) while in captivity and were ultrasounded every 2 – 5 days to assess follicle/egg development. Females that had only follicles or a combination of eggs and follicles were held in a completely aquatic tank (**Figure 1a**.). Once only shelled eggs were visible in the ultrasound images and could be easily felt by palpation, females were placed in a tank system consisting of an aquatic tank connected by a bridge to a tank filled with construction sand (**Figure 1b**.). The sand filled tank was meant to offer a "natural" habitat for females to construct nests. Five females made nests in the sand tank. All other females were induced to lay eggs using oxytocin. Females were brought into the laboratory and injected with 2 units of oxytocin/100 g of body weight (Tucker et al. 2007). They were then placed in a plastic bin of water individually so when eggs were laid they were not accidentally crushed by the female terrapin. Females were injected every 2 hours, up to 3 times/day until all eggs were collected (as determined through ultrasound). The time from injection to egg-laying varied from 30 minutes to multiple days. Once egg-laying began, the time until all eggs were released ranged from 10 minutes to multiple days. Following egg collection, we collected a tail tip for genetic analysis from each female terrapin and they were all released at the site of capture. Each clutch of eggs collected from gravid female diamondback terrapins was measured (length and width in mm) and weighed (grams), buried halfway in Hatchrite nesting substrate in a plastic container and placed in one of two incubators.

Over the course of three nesting seasons (2014-2016), 206 female terrapins were examined for signs of follicles and eggs. In total, 1,089 eggs were collected from 2014 – 2016 from 154 clutches. Mean clutch size was 7.1 eggs/clutch (range 1-17 eggs/clutch) and mean mass of eggs was 11.0 g. Mean egg mass by clutch and number of eggs in clutch were both positively correlated with increasing female mass (**Figure 2**). Mean female mass was 886.7 g. The smallest female that produced viable eggs was 515 g.

In the first year of the study (2014) sampling for adult female terrapins was focused on in-water collection using trammel nets in the Charleston Harbor estuarine system, a method we knew to be effective at collecting adult terrapins during mating and nesting seasons. However, we were also able to collect two clutches of eggs from females that were captured on land. With the assumption that those females collected on land during nesting season were either on their way to nest or returning from nesting and knowing that we could not definitively determine the stage of the eggs in females that we caught in the water other than through subjective palpation of eggs, we began to collect female terrapins both in the water and on land to allow for those comparisons (see Objective 2).

<u>Objective 2 (Years 1 – 3):</u> Incubate eggs at varying temperature ranges in order to produce 50% male hatchlings and 50% female hatchlings. Evaluate hatching success of eggs and growth of hatchlings collected from terrapins in the water vs. on land and from clutches collected via induction with oxytocin vs. clutches laid volitionally (naturally).

During the 2014-2016 mating/nesting season (May through July), viable clutches from 130 diamondback terrapin females captured in trammel nets and viable clutches from 24 land-captured terrapins were collected. In 2014 and 2015 all females captured in the Charleston Harbor area were examined for signs of egg development. In 2016 the focus was to increase numbers of gravid female terrapins on land; therefore only 10 terrapins collected by net and determined to have eggs upon palpation were retained. The ratio of gravid females to total females captured in water for 2016 was not enumerated. All terrapins were captured in the Charleston Harbor system, which includes sites in the Ashley River, Wando River and Charleston Harbor proper. Female terrapins were captured both during routine sampling efforts by Inshore Fisheries (targeting recreationally important species of fishes) as well as during terrapin-targeted sampling trips. Most females collected on land were from Plum Island, Charleston, SC as they made their overland movement in search of suitable nesting habitat; several other females were captured on-land in the Charleston area.

Each clutch of eggs collected from gravid female diamondback terrapins was assigned to one of two incubators. One incubator was set at 27^{o}C to produce male hatchlings and the other was set to incubate at 31^{o}C to produce female hatchlings. Eggs began hatching in 42-50 days in the female incubator (89 clutches, mean 46.6 days) set to the higher temperature and in 55- 64 days in the male incubator (84 clutches, mean – 59.7 days). Overall hatching success for all eggs and all years was 88.2 %. There was no significant difference between hatching success of male (89.9%) vs. female (86.8%) incubator. All hatchlings were measured once their yolk was fully absorbed and their plastron healed (carapace length, plastron length, carapace width, depth and mass). Male terrapins were significantly larger than females at hatching in all metrics ($p<0.05$; **Table 1**).

We compared the difference in hatching success of eggs collected from females harvested in the water vs. those captured on land (**Table 2a**.). The advantage to capturing females in the water is the abundance and efficiency of the capture method compared to the lower frequency of encounter with a female on land and the greater amount of time spent in land collection. In addition, we began to investigate potential differences in hatching success of eggs collected from females after they were induced with oxytocin compared to those that nested volitionally in the “nesting tank” (**Figure 1b; Table 2b**.). A one-way ANOVA (all years pooled) did not detect significant differences in hatching success of clutches collected from females on land vs. in water ($n=139$, $p=0.1425$) nor were there differences in hatching of clutches collected from induction vs. volitional (natural) nesting ($n=139$, $p=0.7743$). Clutches that were entirely unsuccessful and showed no signs of development were eliminated from all analyses. Often these clutches would begin to grow mold and since we could not determine if they had been successfully fertilized they were not included in the model. These are important findings because there is the potential to "headstart" diamondback terrapins in a cost and time-effective manner. The large number of hatchlings able to be produced through these methods could potentially be used to supplement depleted populations and provide terrapins at the hatchling and juvenile life stages to further study on these critical life stages that are extremely cryptic in the wild. During the course of this study 638 terrapins (411 headstarts, 227 hatchlings) were released back into the Charleston Harbor System (**Table 3**).

Significant deviations: Trammel netting was used as the primary method of collection, either deployed parallel to the marsh or set and retrieved in a manner similar to a seine net. The idea of using silt fence/pitfall trap arrays was abandoned early in the study (2014) due to better catch efficiency from trammel net captures and several failed attempts due to extremely high tides, winds and significant storm events. Land-based capture was utilized with the cooperation of the Charleston Water System location at Plum Island where females nest annually and through various other encounters with nesting females in the Charleston area. This change in methods from all land-based capture to a combination of land and water based capture allowed us to make important comparisons in hatching success of these methods. Given that water capture is considerably more time and cost effective, this proved to be a valuable investigation.

Objective 3 (Year 1 - 2): Evaluate the feasibility of culturing diamondback terrapins by altering diet to determine optimal culture growth.

Accomplishments: The effect of three different diets (2 commercially produced pellet types, 1 natural diet) on terrapin growth were evaluated from October 2014 through April 2015. One hundred and eighty hatchlings (90 females, 90 males) were selected for the feed study in early October based on size (no significant difference in hatchling size). Sixty individuals were randomly assigned to each treatment with equal female/male representation. Individuals are identified by a unique numeric identifier written on the carapace with oil-based markers in three different colors based on diet assignment. Prior to the start of the study, all terrapins were acclimated to a pellet or natural diet by offering only one food choice per feeding; in this way we ensured that all animals were feeding at the beginning of the study no matter which feed type they were assigned. Terrapins were housed in seven "raceway" tanks that included low salinity recirculating well water (5-10 ppt), plastic plants for shelter, and basking platforms. They were assigned weekly to one of seven tanks in order to minimize tank effect due to physical or behavioral interactions. Terrapins were fed 4 times per week in separate feeding bins (15 terrapins/bin) by diet treatment. Enough food was offered so terrapins could feed to satiation over the period of 90 – 120 minutes. Diets were either ZooMed (4 mm), Mazuri (4 mm) or a natural diet of fileted and skinned Atlantic croaker (*Micropogonias undulatus*) and spot (*Leiostomus xanthurus*). All turtles were measured (carapace length, carapace width, plastron length, mass) at the beginning of the experiment and monthly to track growth.

Overall, from October 2014 to April 2015, we observed significant differences in growth between the 3 diets ($F_{3,1417}$=717.994, R^2=0.603, p=<0.0001; **Figure 3**). Monthly growth measurements began to show statistical differences starting in January 2015 ($F_{2,177}$=4.309, R^2=0.047, p=0.015). At that time, turtles fed the two pelleted diets (ZooMed and Mazuri) began to diverge from the turtles fed a diet of fish (**Figure 4**). By the conclusion of our study, April 2015, turtles fed either pelleted diet were significantly larger and more active than those fed the fish diet (**Figure 4**). These findings result in two major conclusions: 1) Diamondback terrapins require a varied diet. We know from previous diet studies that diamondback terrapins not only eat fish but that they also eat many small estuarine organisms, including fiddler crabs, periwinkle and mud snails and other benthic invertebrates (Tucker et al. 1995). So, while this is not a surprising result, this small study indicates that, for the purposes of headstarting (accelerating growth as compared to natural conditions by holding animals in stable conditions conducive to maximum growth), (1) a "natural" diet should consist of a combination of several organisms more comparable to the diet of a wild turtle. (2) Commercial pelleted diets are acceptable diets to use for headstarting diamondback terrapins. The pelleted diets we used resulted in above average growth trajectories and a more efficient use of project money and time when compared to using a more "natural" diet.

After April 2015, a portion of terrapins were retained in the laboratory and continued to feed on the Mazuri Aquatic turtle diet. In June of 2016, 24 female turtles hatched from the 2014 yearclass were released into a 0.1-hectare pond (0.25 acres) at the Waddell Mariculture Center in Bluffton, SC. Terrapins in the pond were held at a salinity of 8 – 18 ppt. Salinity was affected by temperature and rainfall but could be maintained by the addition of water from the Colleton River (25-30 ppt) or water from a freshwater well (7-8 ppt). In September 2016 the pond was partially drained and 16 of the 24 females were captured, weighed and measured. In August 2016 one of the terrapins was found dead and partially predated upon in the bottom of an adjacent pond. The seven remaining terrapins that were unaccounted for could merely have avoided capture by burying in the earthen pond bottom. The growth of these juveniles from hatching in the summer of 2014 to September 2016 is shown in **Figure 5**. **Figure 6** shows the change in mass of terrapins from the last measurement in June of 2016 before release into the pond and three months after release in September 2016 (overall positive net growth).

<u>Significant deviations:</u> None.

<u>Objective 4 (Year 2):</u> Continue to track "headstarted" terrapins (2014-year class) through the winter to identify winter hibernacula habitat of juvenile terrapins.

<u>Accomplishments</u>: Six diamondback terrapins were affixed with radio transmitters and released May 2015. After release, poor conditions—including extremely high tides and storms—prevented us from immediately tracking individuals. As a result of the large tides, individuals were thought to disperse further than usual and have become difficult to locate. These six terrapins have not been relocated.

<u>Significant deviations:</u> Due to the difficulty in finding the original six individuals affixed with transmitters, and the extremely large tides resulting from the abnormal rainfall amounts in Charleston this year, we have not released anymore individuals with transmitters at this time. Instead we will attempt to answer the question of preferred overwintering habitat of yearlings by including them in our Year 3 objective below.

<u>Objective 5 (Year 3):</u> Assess the overwintering habitat preference of age 0 terrapins.

<u>Accomplishments</u>: In October 2015, a pen with a 500 ft. perimeter fence encompassing 5 high marsh microhabitats, was constructed in the marsh in Grice Cove, Charleston, SC. Also, 186 hatchlings from 2015-year class and 17 yearlings from the 2014-year class were equipped with a PIT tag. Individuals were

released in the high marsh enclosure mid-November and allowed to move until winter. The pen was checked every 2-3 weeks to scan for terrapins and investigate activity. Raccoon tracks were located following the perimeter of the fence line. The remains of two yearling terrapins were discovered at the base of a cedar tree at the highest portion of the cage in January 2016. Two wildlife cameras were deployed in February to identify specific predator behavior, but no conclusive evidence was found. On February 4, 2016 an extensive search of the pen was conducted and 19 PIT tags were recovered from inside of the pen. Some tags were found near hatchling remains and so it is assumed that these 19 hatchlings were almost certainly predated upon. No terrapins were recaptured alive. Unaccounted for animals were either predated upon or were able to avoid detection if they were buried deeply enough in the plough mud.

Significant deviations: No terrapins were recaptured alive. Unaccounted for animals were either predated upon or were able to avoid detection if they were buried deeply enough in the plough mud. A warmer than average winter could have caused terrapins to remain active for longer than anticipated and may have unexpectedly drawn attention from predators. Since no terrapins were recaptured we could not assess habitat preference or growth.

Literature Cited:

Tucker, A.D., N.N. Fitzsimmons and J.W. Gibbons. 1995. Resources Partitioning by the Estuarine Turtle *Malaclemys terrapin*: Trophic, Spatial, and Temporal Foraging Constraints. Herpetologica 51(2):167-181.

Tucker, J.K., D.L. Thomas and J. Rose. 2007. Oxytocin Dosage in Turtles. Chelonian Conservation and Biology 6(2):321-324.

Estimated Federal Cost: $176,048 to date (amount spent through 9/30/2016, not all charges have posted).

Recommendations: Further study of cryptic life stages of diamondback terrapins, in particular hatchling and juvenile stages, can be accomplished through the use of eggs collected from wild females and reared in a hatchery setting. Terrapins “headstarted” in a laboratory setting until they near size at maturity may be used to assess the viability of these individuals in the reproductive population. While it is clear that individuals allowed to attain a greater size before release into the wild (headstarting) will better avoid predation, we don't know if an increased growth trajectory will allow these animals to enter the reproductive population at a younger age. Also, if they do reach maturity sooner, will they produce high quality viable offspring? These animals serve a dual role in that we can gain important biological knowledge to better manage sensitive populations and then, upon completion of projects, these animals can be released back to natal estuaries helping to supplement depleted populations.

Figures and Tables

Figure 1 a & b. Tank systems that housed gravid female terrapins before egg collection. Females that only had follicles or a mix of follicles and eggs were kept in an aquatic habitat and fed a natural diet until all eggs were sufficiently calcified (a). The bottom photographs (b) show the nesting tank system where females were kept once they were ready for egg collection. A bridge connecting the aquatic side to a tank filled with construction sand offered an area for females to nest volitionally before being induced.

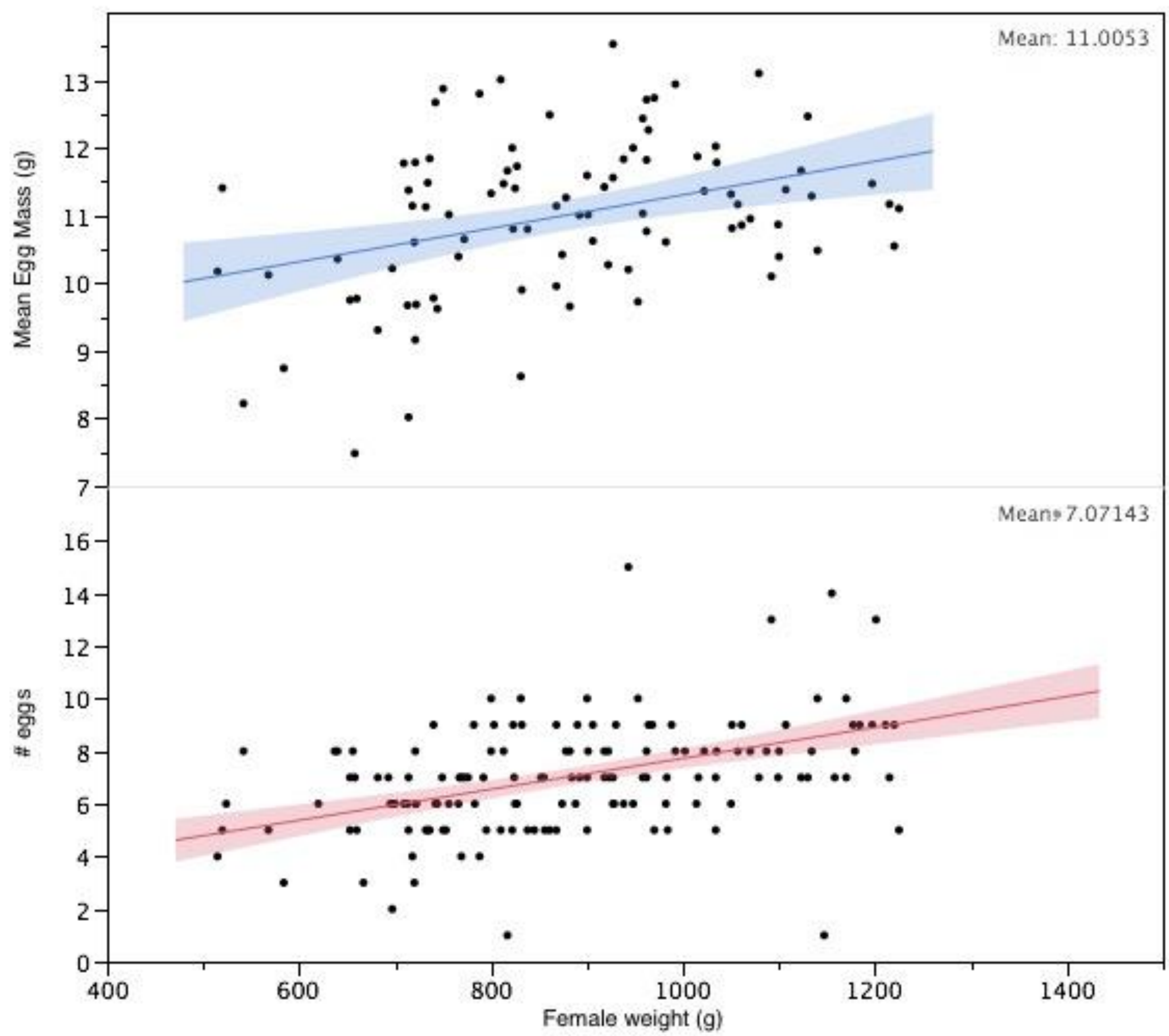

Figure 2. Mean egg mass (g) by clutch and number of eggs per clutch are both significantly greater (P=0.0005 and P<0.0001 respectively) with increasing female mass (g). Only 2015 and 2016 data considered in the mean egg mass since not all eggs were weighed immediately upon laying in 2014. Shaded areas represent 95% confidence intervals.

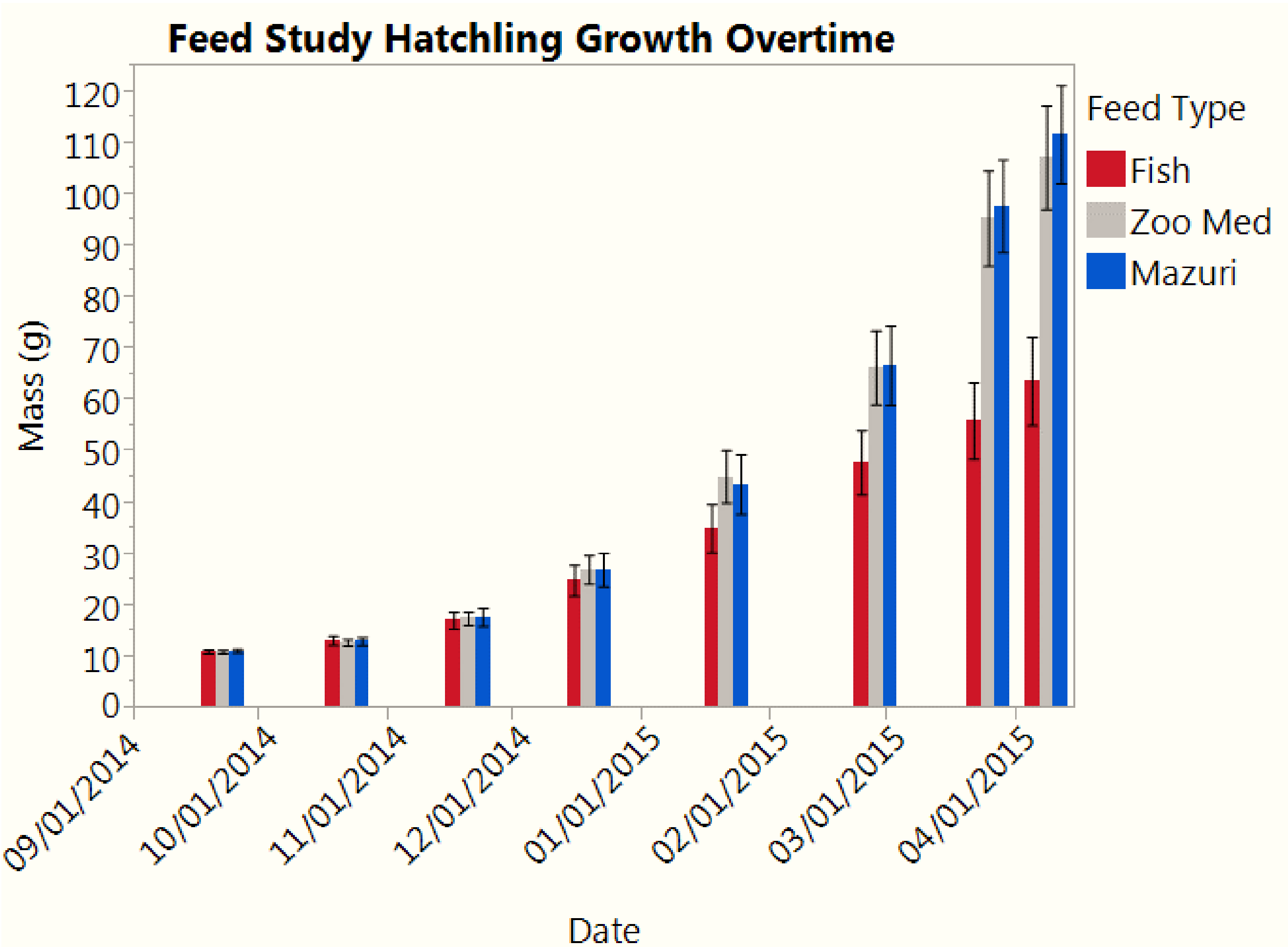

Figure 3. Diamondback terrapin hatchling monthly growth when offered an exclusive diet of one of three diets (ZooMed commercial pellet, Mazuri commercial pellet or fish filets).

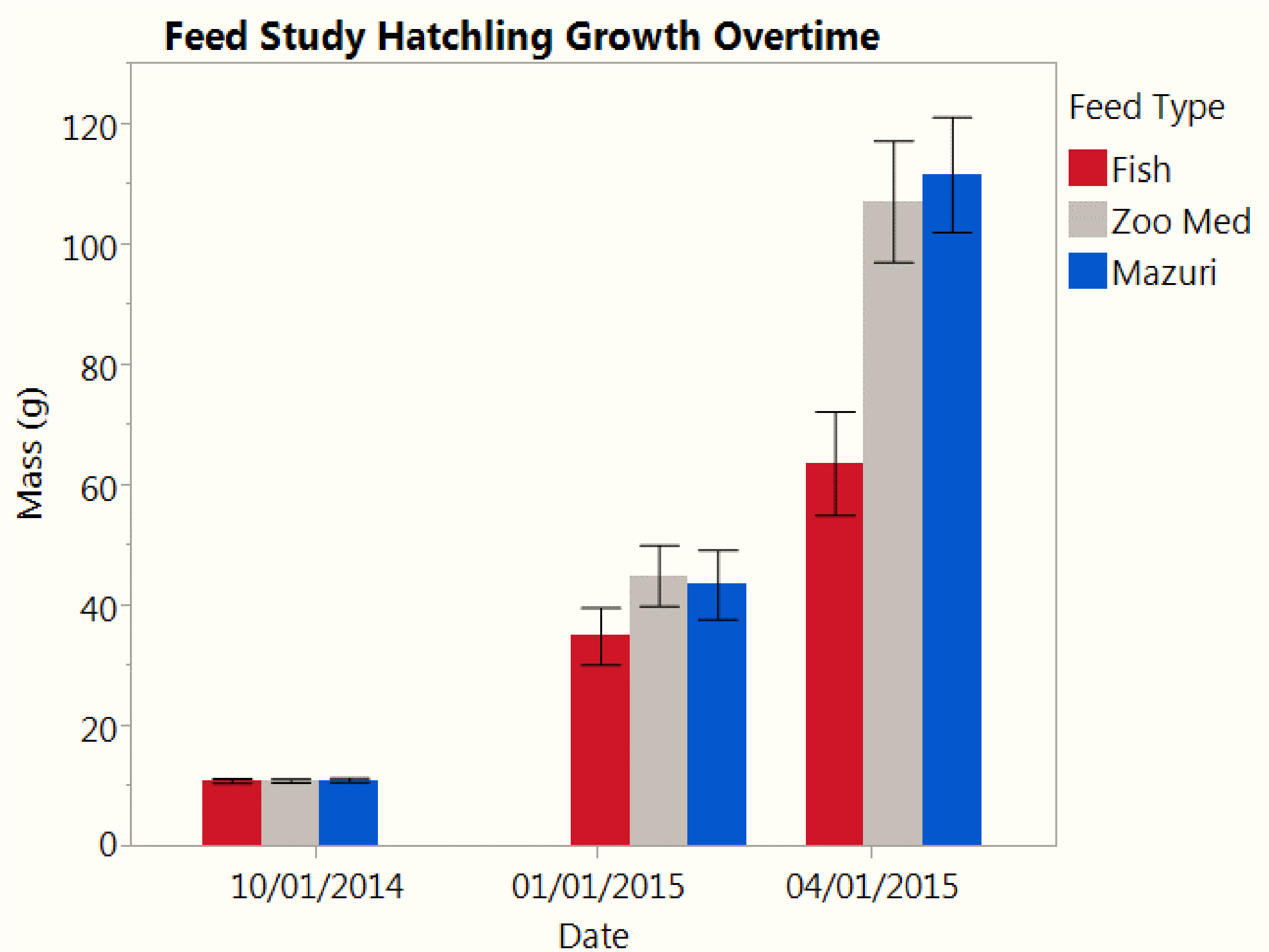

Figure 4. Diamondback terrapin hatchling growth overtime (every 3 months) when offered an exclusive diet of one of three diets (ZooMed commercial pellet, Mazuri commercial pellet or fish filets).

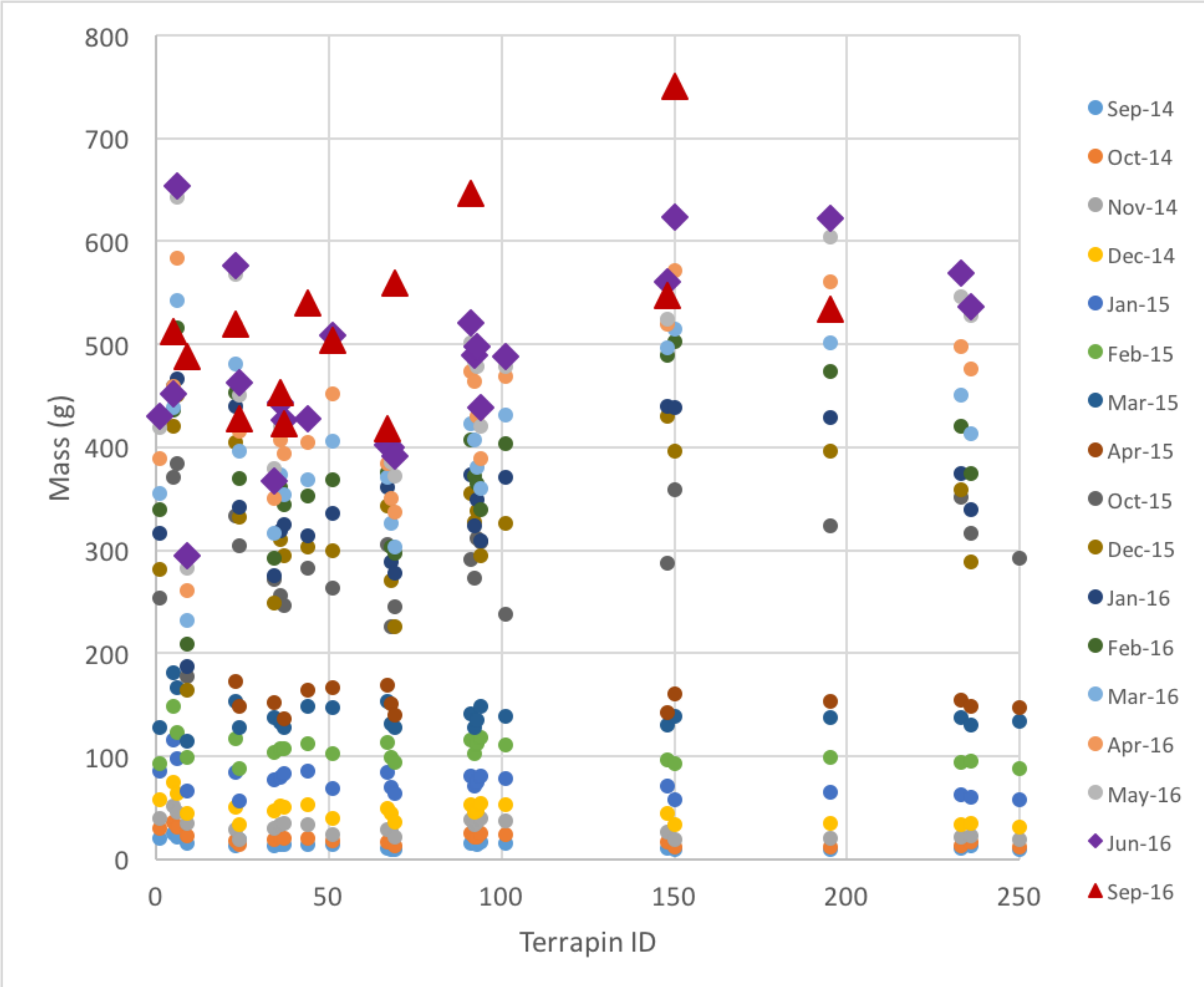

Figure 5. Growth of terrapins hatched in the summer of 2014 and reared in the hatchery until June 2016 when they were released into an earthen bottom culture pond at the Waddell Mariculture Center, Bluffton, SC. At the top of the figure the purple diamonds show the size in June 2016 just before release and the red triangles show the size after 3 months in the pond.

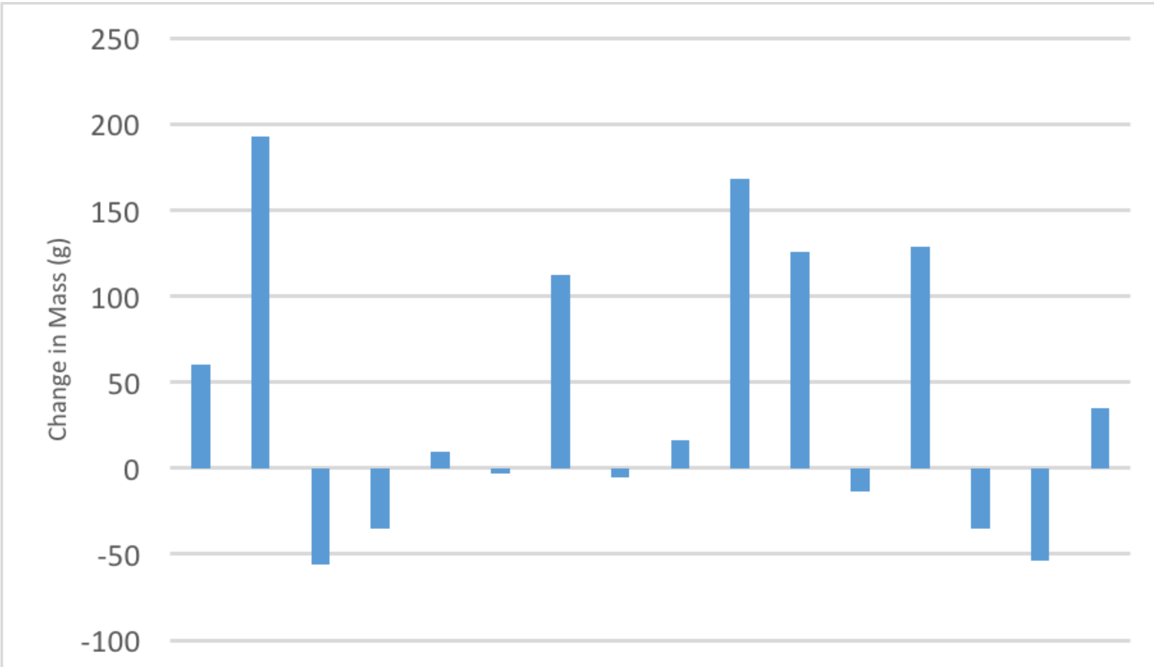

Figure 6. Change in mass of 16 terrapins (YC 2014) raised in hatchery until June 2016. From June 2016 to September 2016 6 terrapins gained > 50 g, 2 terrapins lost > 50 g and the remaining 8 terrapins had a gain/loss < 50 g.

Table 1. Means of hatchling measurements (carapace length, carapace width, depth, plastron length and mass) by sex.

Sex	N	CL (mm)	CW (mm)	Depth (mm)	PL (mm)	Mass (g)
F	454	31.12	26.57	15.91	27.78	8.06
M	303	32.19	27.36	16.27	28.11	8.46

Table 2 a & b. Number of gravid females captured 2014 – 2016 on land or water and associated hatching success (a) and number of gravid females captured 2014 – 2016 that were either induced to collect eggs or those that nested volitionally (natural) and associated hatching success (b).

a.

Year	Sampling Method	# Females	# Eggs	Hatching Success
2014	Land	2	14	0.930
	Water	59	428	0.818
2015	Land	9	47	0.907
	Water	61	422	0.881
2016	Water	10	70	0.964
	Land	13	108	0.887

b.

Year	Egg Collection Method	# Females	# Eggs	Hatching Success
2014	Induction	55	388	0.830
	Natural	6	54	0.744
2015	Induction	66	443	0.882
	Natural	4	26	0.923
2016	Induction	13	107	0.907
	Natural	10	71	0.945

Table 3. Hatchling and Headstart releases by location. Each area is within the Charleston Harbor estuarine system.

Location	Size	N	Total
Charleston Harbor	Hatchlings	187	406
	Headstarts	219	
Ashley River	Hatchlings	31	196
	Headstarts	165	
Wando River	Hatchlings	9	36
	Headstarts	27	